JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., JANUARY 20, 1919 No. 3

VARIATIONS AND MODE OF SECRETION OF MILK SOLIDS[1]

By JOHN W. GOWEN

Assistant Biologist, Biological Laboratory, Maine Agricultural Experiment Station

INTRODUCTION

The chief contributors to the subject of the physiology of milk formation in the mammary gland have chosen as the foundation for their theories the grounds of analogy with the mode of formation of the secretion in the two types of glands, sebaceous and salivary, and as positive evidence have cited histological studies of the mammary tissue. On such grounds the conclusions are likely to be weak. As yet there has been little attempt to use the store of accurate mathematical data on the composition and variation of the milk constituents for the analysis of this problem.

The investigation reported in this paper is an attempt to analyze the variations and associations of the constituents of Holstein-Friesian milk to furnish definite mathematical evidence bearing on the problem of the kind of mechanism liberating these constituents to form the fluid known as milk. The specific problems and the viewpoints taken in this paper may be best understood by considering the natural divisions into which milk secretion falls. The mammary functions may be divided into two main divisions. The first of these has to do with the formation of the materials of milk before the constituents are finally brought together as milk The second has to do with the release of the complete product, milk. The first of these needs only concern us. This problem may be again narrowed to exclude the genetic differences which most certainly exist between individual cows. Given the constituents of cow's milk, our problem is thus limited to the manner in which these constituents are released into the milk ducts. Is it through the secretory action of the gland cells or is it through the destruction of the whole or a part of these cells? Toward the analysis of this problem and related problems the data on the variations and associations of the constituents of cow's milk have been collected. The special problems bearing on the question will be discussed in connection with the data of the later sections of the paper.

[1] Papers from the Biological Laboratory of the Maine Agricultural Station No. 121. This paper is the fourth of a series of studies on milk now being conducted in the Biological Laboratory of the Maine Station.

MATERIAL AND METHODS

The Holstein-Friesian Association (*15*)[1] has, as part of its semiofficial advanced registry work, collected a considerable amount of data on the yearly production of Holstein-Friesian cows. The majority of these records contain the following data: The name, advanced registry number, and herd-book number are given, together with the volume where the last entry record was made, as the requirements of the semiofficial test include the making of the 7-day official test. The rest of the animal's record includes age at calving, length of record, weight of milk, percentage of butter fat and total weight of butter. Some of these records, fewer than we could desire but still far more ample than those of any other known breed, give the total solids for the milk produced. These records will furnish the material for the analyses of the problems previously indicated.

The objection may be raised that these data are not accurate, since they are taken from this type of record. It is realized that there may be some justice in the criticism; yet the inaccuracy should not be magnified. Any advanced registry system must be subject to all the criticism that may be brought against these particular records. Criticism can not be made on the ground of conscious inaccuracy due to poor management on the part of the association officials, as every record is carefully checked for inaccuracies. Each record is under oath as to its accuracy by both parties, the tester, and the owner. The only justifiable criticisms which can be brought are those of the personal equation type, errors from the variation in the values as read by two different men. These errors can not at any time be very great. They constitute that group of errors which are as likely to go one way as another—that is, they should counterbalance.

The methods used are, in general, those of any adequate statistical treatment of a quantitative subject. The constants for the distributions, means, standard deviations, and coefficients of variations are calculated by the usual formula for grouped distributions and without the use of Sheppard (*27*) correction. The correlations are calculated from the correlation surfaces by the usual Bravais formula. The necessity of correcting for the effect of age and quantity of milk in the comparison with the amount of butter fat and solids-not-fat have made necessary the use of partial correlation coefficient to measure such association for a constant value of the disturbing variables. These constants have been calculated from the ordinary correlations by the method devised by Pearson (*21*).

VARIATION OF MILK, BUTTER FAT, AND SOLIDS-NOT-FAT

Some study of the variation of the milk and butter fat have been made, notably those by Gavin (*11*), Vigor (*34*), and Pearl (*20*), where definite variation constants have been determined. These studies in-

[1] Reference is made by number (italic) to "Literature cited", p. 99–102.

clude chiefly the determination of the relation of this milk and butter fat as it varies within itself and as it varies with age. They do not consider the relation of the variation of the other solids; in fact, the available material has been wanting. Such variation constants are highly desirable; in reality, indispensable for the succeeding studies that are to follow.

These Holstein-Friesian data are exceptional in that they are accurate data taken from one breed under the best of conditions. The material as tabled is taken from volumes 18 to 28 of the Advanced Registry of the Holstein-Friesian Association (*15*) for their semiofficial year records. All of the records for milk, total solids, butter fat, and solids-not-fat are for 365 days. The constants are calculated from the grouped frequencies of the formed correlation tables (IV to IX). It is evident from the correlation table for age and percentage of solids-not-fat that one error probably exists in the records either from a typographical cause or from an error in the determination. I shall therefore give the constants both for this animal included and for the distribution where it is omitted. Table I shows the means, standard deviations, and coefficients of variation of the variates. The two values of some of the constants in this table are, first, those for the whole population of the Holstein-Friesian milch cows, and second, those for the population which also records the values for the total solids.

TABLE I.—*Fundamental constants for Holstein-Friesian mean production of milk*

Character.	Number of individuals.	Mean.	Standard deviation.	Coefficient of variation
Amount of milk.........pounds..	1,387	15,417.44 ± 67.7667	3,741.59 ± 47.9111	24.268 ± 0.3285
Do.....................do..	334	15,149.70 ± 111.5272	3,016.18 ± 78.7223	19.909 ± .5398
Age.......................years.	1,387	4.05 ± .0369	2.04 ± .0261	50.371 ± .7919
Do.....................do....	334	4.49 ± .0838	2.27 ± .0592	50.556 ± 1.6218
Butter fat...............pounds..	335	515.37 ± 3.9956	108.43 ± 2.8256	21.039 ± .5720
Do.....................do....	1,387	528.01 ± 2.4334	134.36 ± 1.7204	25.446 ± .3334
Solids-not-fat..............do....	335	1,302.86 ± 9.5828	260.05 ± 6.7769	19.959 ± .5404
Do.....................do....	334	1,301.20 ± 9.5489	258.71 ± 6.7523	19.882 ± .5390
Total solids................do....	335	1,814.78 ± 12.9929	357.59 ± 9.1884	19.429 ± .5250
Do.....................do....	334	1,812.92 ± 12.9723	351.46 ± 9.1731	19.386 ± .5246
Butter fat..............per cent..	1,387	3.441 ± .0058	.318 ± .0040	9.228 ± .1191
Solids-not-fat..............do....	335	8.612 ± .0134	.364 ± .0094	4.221 ± .1101
Do.....................do....	334	8.604 ± .0124	.338 ± .0088	3.923 ± .1015
Total solids................do....	335	12.02 ± .0206	.560 ± .0146	4.657 ± .1215
Do.....................do	334	12.014 ± .0202	.547 ± .0143	4.549 ± .1188

The following facts are easily deducted from Table I: The Holstein-Friesian cows making the semiofficial record have a mean milk production of slightly over 15,000 pounds of milk, containing about 520 pounds of butter fat and 1,300 pounds of solids-not-fat. The ratio of the solids-not-fat to the butter fat is approximately 2.5 to 1. Taken in the form of percentages, the Holstein-Friesian milk contains slightly over 12 per cent of total solids, composed of 3.43 per cent of fat and 8.60 per cent of solids-not-fat. The mean age of the group is slightly more than 4 years. The animals who have total-solids records are, on the average, about ½ year

older than those without such records. This is no doubt due to the progressive tendency to test young animals and to select animals for high production. The animals whose total-solid records were determined are found in the earlier herd books.

It is interesting to compare these values with the milk of other breeds. To facilitate this, Table II has been drawn up. The data for this table have been gathered from many sources, chief among which are the papers of the Agricultural Experiment Stations and the analyses of public chemists. Each tabulated value is, in general, the mean of a considerable number of observations and may be considered close to the true value. Unfortunately it is not possible to obtain the original data so that the other variation constants could be obtained.

TABLE II.—*Mean milk constituents of the different breeds* [a]

Breed.	Total solids.	Fat.	Solids-not-fat.	Ratio of solids-not-fat to butter fat.
	Per cent.	*Per cent.*	*Per cent.*	
Molltaler	13.22	3.86	9.39	2.43 : 1
Blondvich	12.75	3.67	9.09	2.48 : 1
Angler	12.51	3.51	9.00	2.56 : 1
Jeverland	11.86	3.09	8.77	2.83 : 1
Holland	11.54	3.05	8.04	2.63 : 1
East Friesian	11.80	3.09	8.71	2.81 : 1
Lova Rhine	12.12	3.31	8.81	2.66 : 1
Breitenburg	12.34	3.36	8.98	2.67 : 1
Red Holstein	12.07	3.27	8.80	2.69 : 1
Wesermarsch	11.85	3.24	8.61	2.65 : 1
Schwyz	12.76	3.60	9.16	2.52 : 1
Simmental	13.27	4.05	9.22	2.28 : 1
Westerwold	12.99	3.79	9.20	2.42 : 1
Glan	13.57	4.16	9.41	2.26 : 1
Alderney	13.00	3.81	9.79	2.57 : 1
Jersey	14.39	5.12	9.27	1.81 : 1
Guernsey	13.61	4.53	9.08	2.00 : 1
Holstein-Friesian	11.78	3.32	8.46	2.55 : 1
Ayrshire	12.46	3.62	8.84	2.44 : 1
Shorthorn	12.61	3.70	8.91	2.41 : 1
Polled Jersey	13.93	4.67	9.26	1.98 : 1
French Canadian	13.32	4.00	9.32	2.33 : 1
Dutch Belted	12.31	3.40	8.91	2.62 : 1
Brown Swiss	12.61	3.62	8.99	2.48 : 1
Red Polled	12.66	3.67	8.99	2.45 : 1
South Devon	12.93	3.72	9.21	2.47 : 1
Kerries	13.10	4.02	9.08	2.26 : 1
Dexter	12.58	3.46	9.11	2.63 : 1
Holstein-Friesian	12.02	3.44	8.60	2.50 : 1

[a] The references to the data combined in this table will be found in the following numbered papers of the "Literature Cited": 1, 7, 10, 12, 14, 19, 24, 30, 35, 36, 39, 40.

These data are not entirely satisfactory, representing, as they do, data collected under a great variety of conditions. This heterogeneity is unfortunate. Two errors are easily discernible: The fat percentage of the Holstein-Friesian is about 0.1 per cent too low, and the fat percentage of the Guernsey from unpublished data on 4,900 animals for a year's test is 4.9 instead of the 4.53 of the above list. However, this is the only

material available where any number of animals are tested for their total solids and percentage of fat. The belief is held that even with these discrepancies the table will give a fair comparative view of the average composition of the milk of the various breeds included. Taken at their face value, the data show that the butter fat percentage in the different breeds varies between 3.05 and 5.12 per cent. Similarly, the total solids are shown to vary between 11.54 and 14.39 and the solids-not-fat between 8.04 and 9.79. This would make the average composition of the Holstein-Friesian breed rather lower than most of the other breeds, both in the percentage of butter fat and in the total solids. If we consider now the ratio of the solids-not-fat to the butter fat when the milk is constant, the values of the ratios run between 1.8 and 2.81. This means that the breed considered in the data has a high proportion of solids-not-fat. There appears to be an association between the percentage of fat characteristic of the breed and the content of the solids-not-fat carried in the milk—that is, the Jerseys, with their high fat percentage, also have an increased amount of the solids-not-fat over the other breeds, and the Holland, one of the lowest breeds, also has the lowest amount of solids-not-fat. This increase does not go up in direct proportion to the amount of fat present in the milk, as a glance at the proportion of the two will show. It will remain for a later section to show how these constituents vary within the Holstein-Friesian race.

Data have been tabulated to show the differences in the milk of different species of animals (Table III).

TABLE III.—*Mean milk constituents of different species of animals* [a]

Species.	Total solids.	Fat.	Solids not fat.	Ratio of solids not-fat to butter fat.
	Per cent.	*Per cent.*	*Per cent.*	
Sow	18.51	6.60	11.91	1.8 : 1
Goat	11.80	3.54	8.26	2.3 : 1
Ewe	18.52	7.17	11.35	1.6 : 1
Indian buffalo	16.24	6.77	9.47	1.4 : 1
Bitch	15.89	5.65	10.24	1.8 : 1
Ass	9.72	.90	8.82	9.8 : 1
Mare	10.92	.99	8.93	9.0 : 1
Man	11.78	3.28	8.50	2.6 : 1
Cow	12.01	3.44	8.61	2.5 : 1
Colostrum of cow	22.88	2.30	20.58	8.9 : 1
Colostrum of man	12.91	2.60	10.31	4.0 : 1

[a] The references to the data named in this table will be found in the following numbers of the "Literature cited." 5, 6, 8, 9, 18, 22, 25, 26, 29, 32, 33, 38.

The data given above are open to the same criticism as that in Table II, and are to be taken with the same limitations.

The milk of the different species varies considerably both in its butter-fat content and in its solids-not-fat. The lowest percentage of fat produced is 0.90 per cent, found in the milk of the ass. The milk of the mare corresponds closely to this, 0.99 per cent. The highest percentage of

butter fat is 7.17 per cent, contained in the milk of the ewe, closely followed by that of the Indian buffalo and the sow. The milk of the dairy cow is about intermediate between these two extremes. The colostrum is lower in its fat content than is either of the normal milks of the same species. The solids-not-fat content varies from 8.61 per cent for the cow to 11.91 per cent for the normal milk of the species included in the table. It reaches its highest value in the colostrum, where the cow's milk includes as much as 20.58 per cent. The same general association is also seen in the milk of the different species that are present in the milk of the different breeds—that is, the milk of species containing a low percentage of fat contains proportionately more solids-not-fat than does the species which contains the higher percentage of fat. The species containing a low percentage of fat contains less actual solids-not-fat per hundred pounds of milk than does the species containing a higher percentage of fat.

A survey of Table I shows that the most variable character in the Holstein-Friesians, is the age included in the tests. This has a very high coefficient of variation, the highest shown by any of the measured characters, emphasizing the need of a proper age correction when the amount of milk is to be studied for its hereditary behavior.

The next highest variation coefficient is that for milk (24.3). This is closely followed by the variation coefficients for butter fat, 21.0; solids-not fat, 19.9; and total solids, 19.4 These last four coefficients may be considered as dependent variables and owe part of their variation to variations in other characters. Thus, the large constant of variation for the amount of milk is due in some part to the age differences in the animals included, for, as has already been shown, milk production rises in a logarithmic curve with increasing age. Again, as will be shown later, the relation of the milk constituents to the amount of milk is so close that a large part of their variation may be explained by variations in the amount of milk. As a matter of fact, these data are not needed here, for when the coefficients of variation for the percentages of butter fat, solids-not-fat, and total solids are considered, it is seen that the coefficients are reduced to 9.2, 3.9, and 4.5, respectively, or coefficients which compare rather favorably with physical variables. The high coefficients of the milk solids are thus shown to be due to the variations in amount of milk and not to variations in percentage contents of these constituents.

FACTORS AFFECTING THE COMPOSITION OF MILK

Already some work has been done on factors affecting the composition of milk by Wilson (*37*) and by Pearson (*21-23*). In his studies Wilson attempts to show that the percentage of butter fat is not dependent on the amount of milk. The methods used to draw this conclusion are, according to Pearson, open to criticism on the following grounds: The tail frequencies are clubbed together so that the real correlation can not be

determined, and the means of the separate distributions lead us to a correlation ratio, which, while small, is highly significant in showing the dependence of butter-fat concentration on amount of milk.

These studies limit themselves to the relation of butter fat to the milk. In dealing with this question the association of the percentage solids-not-fat with the quantity of milk produced will also be studied. Tables IV and V show the correlation surfaces for weight of milk and percentage of butter fat and the age of the cow and the percentage of butter fat.

TABLE IV.—*Correlation surface for amount of milk and percentage of butter fat as deduced from individual year records of Holstein-Friesian cows*

Weight of milk (pounds).	Percentage of butter fat. 2.4–2.6	2.6–2.8	2.8–3.0	3.0–3.2	3.2–3.4	3.4–3.6	3.6–3.8	3.8–4.0	4.0–4.2	4.2–4.4	4.4–4.6	4.6–4.8	Total.
7,000– 9,000				3	5	6	5	3	1		1		24
9,000–11,000			1	17	32	25	25	15	4				119
11,000–13,000		2	17	42	66	90	58	17	11	3	1		307
13,000–15,000		4	12	59	96	117	67	30	9	5	1		400
15,000–17,000	1	3	9	66	107	92	49	31	8	5			371
17,000–19,000	2	4	20	55	79	66	48	14	4	1	1	1	295
19,000–21,000		3	7	16	49	50	13	8	3	2	3		154
21,000–23,000		3	7	16	25	24	8	7	2	2	2		96
23,000–25,000		2	3	6	8	4	6	1			2		32
25,000–27,000		1	4	5	4	3	3	3	2				25
27,000–29,000			2	5	1	2	2	1	1	1			15
29,000–31,000				5									5
Total	3	22	82	295	472	479	284	130	45	19	11	1	1,843

TABLE V.—*Correlation surface for age at the beginning of test and percentage of butter fat contained in the milk for the individual records of Holstein-Friesian cows*

Age at test. Yr. mo. Yr. mo.	Percentage of butter fat. 2.4–2.6	2.6–2.8	2.8–3.0	3.0–3.2	3.2–3.4	3.4–3.6	3.6–3.8	3.8–4.0	4.0–4.2	4.2–4.4	4.4–4.6	4.6–4.8	Total.
1 6– 2 0			1	7	12	16	10	3	3	1			53
2 0– 2 6	2	4	11	66	84	91	66	21	6	1	2		354
2 6– 3 0		2	7	23	38	59	38	21	8	4			200
3 0– 3 6		1	4	15	29	25	19	13	1	2	1	1	111
3 6– 4 0		2	8	20	32	28	20	9	2	2			123
4 0– 4 6		1	5	15	17	12	18	7	4		2		81
4 6– 5 0	1	2	6	7	20	23	8	8	1		1		77
5 0– 5 6			5	10	16	20	8	8	2	2	1		72
5 6– 6 0		2	2	14	22	23	13	2	2				80
6 0– 6 6				10	19	13	6	3					51
6 6– 7 0			1	6	17	18	4		3				49
7 0– 7 6		1	1	11	5	17	3		1	1			40
7 6– 8 0				4	5	8	4	2					23
8 0– 8 6				3	8	5	2						18
8 6– 9 0			1	3	5	6		1		1			17
9 0– 9 6		1	1	2	5		4	2					15
9 6–11 0			2	2	3	6	2	1	1				17
11 0–15 0				2	2	1			1				6
Total	3	16	55	220	339	371	225	101	35	14	7	1	1,387

A glance at these tables shows that there is little correlation between butter fat percentage and the weight of milk or age at test for the Holstein-Friesian cows. It seems well before tabulating these coefficients that we consider the effect of increased production in Holstein-Friesian cattle on the percentage of solids-not-fat contained in the milk. Two tables similar to those above are necessary for this comparison. These data are given in Tables VI and VII.

TABLE VI.—*Correlation surface for the amount of milk produced in one year and the percentage of solids not fat contained in the milk of Holstein-Friesian cows*

Weight of milk (pounds).	Percentage of solids-not-fat.																					
	7.0–7.2	7.2–7.4	7.4–7.6	7.6–7.8	7.8–8.0	8.0–8.2	8.2–8.4	8.4–8.6	8.6–8.8	8.8–9.0	9.0–9.2	9.2–9.4	9.4–9.6	9.6–9.8	9.8–10.0	10.0–10.2	10.2–10.4	10.4–10.6	10.6–10.8	10.8–11.0	11.0–11.2	Total.
7,000–9,000									1		1											2
9,000–11,000				1	1		2	5	6	4		1										20
11,000–13,000					1	7	5	10	13	17	9	1										63
13,000–15,000	1				4	7	10	16	24	15	6	2										85
15,000–17,000			1		1	7	10	19	21	10	4										(1)	74
17,000–19,000					3	5	4	18	13	9	4	2		1								59
19,000–21,000						4	2	5	6	3	3											23
21,000–23,000						1	1	2		1	1											6
23,000–25,000								1														1
25,000–27,000								2														2
Total	1		1	1	10	31	34	78	84	59	28	6		1							1	335

TABLE VII.—*Correlation surface for the variables age at test and percentage of solids not fat for the semiofficial year records of Holstein-Freisian cows*

Age at test	Percentage of solids-not-fat.																					
Yr. mo. yr. mo.	7.0–7.2	7.2–7.4	7.4–7.6	7.6–7.8	7.8–8.0	8.0–8.2	8.2–8.4	8.4–8.6	8.6–8.8	8.8–9.0	9.0–9.2	9.2–9.4	9.4–9.6	9.6–9.8	9.8–10.0	10.0–10.2	10.2–10.4	10.4–10.6	10.6–10.8	10.8–11.0	11.0–11.2	Total.
1 6–2 6	1			1		2	7	15	22	18	12	4										82
2 6–3 6					3	6	1	14	18	15	3			1								61
3 6–4 6						4	9	15	14	7	6	1										56
4 6–5 6			1		1	4	6	11	8	10	1	1										43
5 6–6 6					2	5	3	10	9	4	1											34
6 6–7 6					1	5	4	5	4		3											22
7 6–8 6						2	1	4	1	2												10
8 6–9 6					1	2	1	3	5	2	1										(1)	16
9 6–10 6					1	1		1	2		1											6
10 6–11 6					1					1												2
11 6–12 6							2		1													3
Total	1		1	1	10	31	34	78	84	59	28	6		1							1	335

It will be noticed that in both Tables VI and VII there is one value (in parentheses) far removed from the distribution of the other entries. It seems desirable to determine the correlations with this value included and excluded. Consequently, the correlations will be given both with and without it, since it is highly probable that some error has crept into the determination of the solids for this test.

Table VIII gives the values of the correlation coefficients and the correlation ratios, together with the constants to show the approach to linearity of the regression lines.

TABLE VIII.—*Analytical constants for inter-individual variation of the constituents of cow's milk*

Character correlated.	r	η	Σ_m	η^2-r^2
Weight of milk and percentage of butter fat	−0.0977±0.0156	0.1251±0.0155	0.1411	0.0061±0.0014
Age and percentage of butter fat	− .0546± .0181	.1325± .0178	.1917	.0146± .0044
Weight of milk and percentage of solids-not-fat	− .0553± .0367	.1161± .0364	.1857	.0104± .0075
Age and percentage of solids not fat without doubtful observation	− .1612± .0359	.2341± .0348	.3084	.0288± .0124
Weight of milk and percentage of solids-not-fat	− .0659± .0367	.1373± .0362	.2032	.0145± .0089
Age and percentage of solids-not-fat	− .2191± .0351	.2459± .0347	.1883	.0125± .0084

This table shows that the weight of milk produced in a year is negatively correlated with the percentage of butter fat and of solids not fat contained in this milk. In each case the correlation is low, in neither case being as great as −0.1. For the butter fat percentage the correlation −0.0977±0.0156, although low, is highly significant, since the correlation value is 6.2 times its probable error; or, in other words, could be expected from random sampling only once in slightly more than 100,000 times. The correlation for percentage of solids not fat and milk, −0.0553±0.0367, is only about half that for the percentage of butter fat and milk. Further, this correlation can not be considered significant, as it is only 1.5 times its probable error, or about once out of three trials a correlation as great or greater than this due to random sampling would be expected. It will be noted that even where the abnormal observation is eliminated the correlation does not increase to a value where it becomes significant.

The correlation between the percentage of butter fat and age is slight (−0.0546±0.0181) and in the same direction as that of butter fat and milk production—that is, minus. It may possibly be significant, since it is about 3.1 times its probable error. Even if it were significant, however, it would be scarcely detectable except in a large mass of data where statistical methods were applied.

On the other hand, the correlation between age at test and percentage of solids-not-fat is significant, for, with the doubtful observation, the correlation is 4.4 times its probable error, and without this doubtful observation the correlation is 6.2 times the probable error. Furthermore, the difference between the correlation of percentage of butter fat and percentage of solids-not-fat in cow's milk and the age at which the test is made[1] is probably a significant difference. The difference of the correlation of the percentage solids-not-fat and age and the correlation be-

[1] The probable error of the difference is calculated by the usual formula $\pm 0.67449\sqrt{\sigma_1^2+\sigma_2^2}$.

tween the percentage of butter fat and age is slightly over 2.7 times its probable error when the doubtful observation is included in the data. The difference and its probable error for this is 0.1066 ± 0.0400, or 2.7 times its probable error when the doubtful observation is included in the data. The difference and its probable error for this is 0.1066 ± 0.400, or the difference is 2.7 times its probable error. When this doubtful value is not included in the calculations, the difference and its probable error becomes 0.1645 ± 0.0395, or 4.2 times the probable error, a value which certainly represents a greater effect of age on the solids-not-fat content of cow's milk than of age on the butter-fat content of the same milk.

Much the same statement holds for the relation of the percentage of solids-not-fat and weight of milk produced and percentage of the solids-not-fat and age at test. The difference is of the same magnitude as is the difference between percentage of solids-not-fat and percentage of butter fat and age—that is, the difference is only slightly significant if we consider the correlation found in the presence of the doubtful value, and is markedly significant when this value is thrown out of the table.

LINEARITY OF REGRESSION

The analytical constants necessary to test the linearity of regression are given in Table VIII. In every case the correlation ratio is a somewhat larger numerical quantity than the correlation coefficient for the same table. These differences are shown to be of little significance in view of the fact that Σ_m and $\eta^2 - r^2$ are substantially zero. The difference between the correlation ratios and the correlation coefficients are probably not significant. In only one case is the difference $\eta^2 - r^2$ greater than three times the probable error (3), and in this case the difference is only 3.3 times the probable error. For this one case the difference is in all probability not significant. For the other correlations the difference is certainly not significant. It may be concluded, therefore, that the regressions are linear and that the correlation coefficient represents the true correlation.

The following conclusion may be drawn from the above analysis concerning the relations between the constituents of cow's milk and the variables, age at beginning of the year test and amount of milk produced during this year test.

1. As the amount of milk given by the cows in this test increases, the percentage composition of the butter fat in this milk decreases. The amount of this decrease is statistically significant. Considered practically, this fall in butter-fat content could not be easily detected in the small samples usually handled.

2. There is a slightly significant fall of the percentage of butter fat contained in the milk as age advances. This slight fall may, however, be accounted for by the rise in milk production which occurs coincident with

this increase in age. For the partial correlation between percentage of butter fat and age, holding the milk production constant, is 0.0105 ± 0.0181, or there is no significant correlation.

3. The quantity of milk produced for the year is entirely independent of the percentage content of the solids-not-fat, or put in another way, the factor or factors causing high or low milk production are separate and distinct from those causing a high percentage of these constituents in the milk.

4. Age is a prominent factor in bringing about the reduction of the percentage of solids-not-fat. This reduction is not due to differences in the amount of milk, as the milk held constant by the partial correlation method gives practically the same correlation as when the milk production is not considered.

5. Decrease in the amount of milk raises the percentage of butter fat in a greater degree than it affects the percentage of the solids-not-fat.

6. Increased age has a marked effect in reducing the percentage of solids-not-fat. It does not so reduce the percentage of butter fat.

CORRELATION BETWEEN THE BUTTER FAT AND THE SOLIDS-NOT-FAT IN COW'S MILK

This question of the correlation between the butter fat and the solids-not-fat has considerable importance for the problems of the mechanism of milk secretion in the mammary gland. Does this gland secrete a high content of butter fat when it secretes a high content of the other solids—lactose, protein, and ash? Should such an association exist, it becomes evident that the factors leading to a high fat content also lead to a high solids-not-fat content. Taken in connection with other theory to account for the presence of the organic constituents of milk, such a correlation indicates a regulatory mechanism which balances these constituents together in similar proportions in any given individual cow.

Apart from the bearing on the problems of the milk secretion, such a correlation has wider significance. It predicates that the factors in inheritance for this high content of one constituent also transmits the production of high content of the other solids. The problem thus becomes important for the student of inheritance of quantity and quality in cow's milk.

To answer these questions for Holstein-Friesian cows, it is necessary to arrange the data for the yearly records of butter fat in a table of double entry or correlation table. In the arrangement of this table 100-pound intervals were chosen as the basis of division of both the solids-not-fat and butter-fat production of the year-tests. In the first two observations of the butter fat it is necessary to group these between 280 and 300 pounds and calculate accordingly. The results are given in Table IX.

Observation easily shows that these two variates, solids-not-fat and butter fat of cow's milk, are highly correlated. The correlation coeffi-

cient in this case is $r = 0.8991 \pm 0.0071$ and the correlation ratio $\eta = 0.9023 \pm 0.0069$. As in the previous tables, the cell which contained the doubtful observation is indicated by parentheses. Where this doubtful observation is left out of consideration, the correlation rises slightly to $r = 0.9095 \pm 0.0063$, and the correlation ratio is $\eta = 0.9064 \pm 0.0066$.

TABLE IX. *Correlation surface for pounds of solids-not-fat and pounds of butter fat as deduced from individual year records*

Weight of solids not fat (pounds)	Weight of butter fat (pounds). 200–300	300–400	400–500	500–600	600–700	700–800	800–900	900–1,000	Total.
700–800	1	3							4
800–900	1	6	2						9
900–1,000		20	5						25
1,000–1,100		13	28						41
1,100–1,200		2	40	5					47
1,200–1,300			23	24					47
1,300–1,400			5	43	2				50
1,400–1,500			2	33	4				39
1,500–1,600				13	17				30
1,600–1,700				2	16	2			20
1,700–1,800					6	4	1		11
1,800–1,900				(2)		4			6
1,900–2,000						2	1		3
2,000–2,100						1			1
2,100–2,200						1			1
2,200–2,300								1	1
Total	2	44	105	122	45	14	2	1	335

These constituents of cow's milk are shown by these correlations to be highly correlated variates. This correlation can not be accounted for by the regression of solids-not-fat on butter fat, not being a linear regression, as even a glance at the values of the correlation coefficient and the correlation ratio will convince anyone that they are so nearly the same in value as to make it certain that the regressions are linear. Consequently it does not seem necessary to calculate the customary constants for this linearity, as both Σ_m and $\eta^2 - r^2$ would be negligible quantities. This establishes the conclusion finally that butter fat and solids-not-fat contained in cow's milk are correlated variates. This correlation being positive, a rise in the amount of either constituent also means a rise in the other.

Part of the correlation between the butter fat and solids-not-fat may be due to the rise in the amount of milk of the different individual cows. For the problem of the mechanism of the secretion of these constituents this is of especial importance. Tables X and XI give the correlation surfaces for the variables.

TABLE X.—*Correlation surface for amount of milk and amount of butter fat as deduced from individual year records of Holstein-Friesian cows*

Weight of butter fat (pounds).	Weight of milk (pounds).										
	7,000–9,000.	9,000–11,000.	11,000–13,000.	13,000–15,000.	15,000–17,000.	17,000–19,000.	19,000–21,000.	21,000–23,000.	23,000–25,000.	25,000–27,000.	Total.
280–300		2									2
300–400	2	14	25	2	1						44
400–500		4	36	56	8	1					105
500–600			2	27	(59)	32	2				122
600–700					6	25	13	1			45
700–800						1	7	4	1	1	14
800–900							1	1			2
900–1,000										1	1
Total	2	20	63	85	74	59	23	6	1	2	335

TABLE XI.—*Correlation surface for amount of milk and solids not fat as deduced from individual year records of Holstein-Friesian cows*

Weight of solids-not-fat (pounds).	Weight of milk (pounds).										
	7,000–9,000.	9,000–11,000.	11,000–13,000.	13,000–15,000.	15,000–17,000.	17,000–19,000.	19,000–21,000.	21,000–23,000.	23,000–25,000.	25,000–27,000.	Total.
700–800	2	2									4
800–900		9									9
900–1,000		9	16								25
1,000–1,100			33	8							41
1,100–1,200			14	32	1						47
1,200–1,300				37	10						47
1,300–1,400				8	38	4					50
1,400–1,500					22	17					39
1,500–1,600					2	25	3				30
1,600–1,700						13	7				20
1,700–1,800							10	1			11
1,800–1,900					(1)		3	2			6
1,900–2,000								3			3
2,000–2,100									1		1
2,100–2,200										1	1
2,200–2,300										1	1
Total	2	20	63	85	74	59	23	6	1	2	335

These tables show that the variables butter fat and solids-not-fat are highly correlated with the amount of milk produced. The correlation coefficient for butter fat and milk is $r=0.8644 \pm 0.0093$ and the correlation ratio $\eta=0.8638 \pm 0.0093$. The correlation coefficient for solids-not-fat and milk is $r=0.9497 \pm 0.0036$ and the correlation ratio is $\eta=0.9484 \pm 0.0037$. As in the preceding case, these variates are

highly correlated. The regressions are so obviously linear that it does not seem necessary to calculate any of the customary constants for determining this, other than the correlation ratio given above.

In order to obtain the coefficients to measure the relation between the butter fat and solids-not-fat for a constant production of milk, it is necessary to resort to correlation of the first order given by the formula of Pearsons (*21-23*).

$$r_{12.3} = \frac{r_{12} - r_{13} r_{23}}{\sqrt{1 - r_{13}}\sqrt{1 - r_{23}}}$$

When the correlation between the butter fat and solids-not-fat for a constant quantity of milk is thus measured, it is found that the partial correlation coefficient is $r_{12 \cdot 3} = 0.4964 \pm 0.0278$ for the case where the doubtful observation (shown in parenthesis) is included in the calculations and is $r_{12 \cdot 3} = 0.5635 \pm 0.0252$ where this doubtful observation is not included. These correlations show that the production by the mammary gland of butter fat and of solids-not-fat are correlated functions. This correlation being plus it means that an increase in the liberation of either constituent of cow's milk means a coincident increase in the other.

This conclusion is important, as it shows that the factors responsible for the increase in the content of butter fat for a given volume of milk are in a high degree responsible for the increase in the solids-not-fat content in this same milk. It means that the physiology of the mammary gland in elaborating the milk solids is such that the release of a certain amount of butter fat to the milk also releases a proportionate amount of solids-not-fat. In order to account for this correlation, it is necessary to explain how the mammary gland sorts out the different elements into the milk to give the proportion of the butter fat and solids-not-fat. This question refers itself back to the fundamental one of how milk is secreted.

DIURNAL VARIATION OF THE CONSTITUENTS IN COW'S MILK

Before discussing the direct bearing of these data on the problem of the milk secretion a few more important data must be presented. It is a well-known fact that the evening milk of a cow is, in general, higher in butter fat than the morning milk. The relation of the evening and morning milk for solids-not-fat is not so well known. Table XII gives this relation for two groups of cows: Those in the first half of their lactation period and those in the last half.

This table shows the variation between morning and evening milk in the percentage composition of the same individual cow. The sample of two consecutive mornings' milk were made, and aliquot parts of each composited for analysis. The same holds true for the evening milk. Each percentage may therefore be said to represent the mean

between two days of lactation for morning and for evening milk. The milkings begin in the morning at 4.45 and in the evening at 3.45. The interval therefore is shorter between the morning-to-evening milking.

TABLE XII.—*Daily variation of the constituents of cow's milk* [a]

Cow No.	First half of lactation.				Last half of lactation.			
	Morning.		Evening.		Morning.		Evening.	
	Fat.	Solids-not-fat.	Fat.	Solids-not-fat.	Fat.	Solids-not-fat.	Fat.	Solids-not-fat.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	4.70	8.35	4.80	8.36	4.20	8.19	4.90	8.76
2	4.00	8.49	5.00	8.60	4.40	8.98	4.80	9.18
3	3.00	8.48	3.30	8.77	4.40	9.60	6.20	9.55
4	5.20	8.55	7.20	8.59	3.20	8.03	4.10	7.95
5	3.40	8.76	4.50	8.64	6.80	10.00	8.00	9.96
6	4.20	8.79	4.80	8.85	4.90	8.95	5.20	9.12
7	3.30	8.34	4.00	8.41	3.50	8.25	3.80	8.47
8	4.10	9.05	4.40	9.29	3.60	8.41	4.20	8.11
9	3.90	9.00	4.80	9.01	4.50	8.58	4.90	9.00
Average	4.078	8.646	4.756	8.724	4.389	8.777	5.122	8.90

[a] The author is indebted to the Chemistry Laboratory of this Station for the careful analysis given in Table XII.

The average composition of morning milk in the first half of the lactation is seen by Table XII to be 4.078 per cent butter fat and 8.646 per cent solids-not-fat. The average composition of evening milk is 4.756 per cent fat and 8.724 per cent solids-not-fat. Thus, the butter-fat constituent increases markedly in the evening milk over that of the morning. The increase of the solids-not-fat is not as marked, although a slight increase does occur. The significance of this increase is supported further by the same kind of relationship exhibited by the morning and evening milk of the cows in late lactation. The numbers are not large, but the consistency of the increase composition of butter fat of the evening milk over that of the morning leads to the conclusion that this relation is certainly significant. For the increase of solids-not-fat the case is not so clear. It is possible that this increase is slightly significant, but this seems doubtful. In no case is this rise of the solids-not-fat as great as that of the butter fat. This, taken in consideration with the fact that the solids-not-fat are more than twice as great in amount as the butter fat, establishes the conclusion that the butter-fat composition of milk is affected to a much greater extent by these different times of milking than are the other solids.

This conclusion is further emphasized by the work of Ingle (*16*) on the same question. In the mixed milk of a herd of 23 animals milk for a period of 18 weeks the average composition of the morning milk

was 2.97 per cent butter fat and 8.87 per cent solids-not-fat, and the evening milk was 4.31 per cent butter fat and 8.86 per cent solids-not-fat.

The significance of these facts as above established on the problem of the mode of liberation of the constituents into cow's milk has been overlooked. Before 1850 the prevailing opinion held that the milk solids were filtered out by the mammary gland from the blood serum. This view was shown to be incorrect by the fact that lactose is not present in the blood and the fat percentage of the serum is not sufficient to account for the fat in a single milking. To replace this old theory, three major hypotheses have been put forth to account for the secretion of the mammary gland:

(1) Cells of the gland break loose bodily and disintegrate in the alveoli to form the milk solids.

(2) The portion of the cells toward the alveoli becomes loaded with solids, breaks loose from the basal portion, and disintegrates to form the milk solids.

(3) The cells of the mammary gland secrete the materials of the milk solids without themselves breaking down.

In opposition to the first theory, it may be said that no such extensive cell multiplication is witnessed in the mammary gland as would be necessary to replace the cell destruction called for on the theory. This disintegration, as pointed out by Heidenhain (*13*) for the milk produced by some cows in one day would require the replacing of all cells in the udder at least five times a day, a replacement of cells unprecedented in our knowledge of cell division.

The second theory, suggested by Langer and ably supported by Heidenhain (*13*), Steinhaus (*28*), and Brouha (*4*), lays its foundation on histological evidence. According to this evidence, the gland cells lengthen out into the lumen of the alveoli. The projecting ends of these cells become loaded with nutrients similar to milk solids. These projecting ends disintegrate to allow the escape of these solids. The basal portions, including a nucleus, are left to rebuild the cell and to enable it to repeat the process. Steinhaus says that, in order to support this rebuilding, mitotic divisions are frequent, and that the daughter nuclei which lie on the outer portion of the cell often degenerate.

The third theory lays its stress on analogy with the other secretory glands without other supporting evidence than the negative evidence of Bertkau (*3*), who says the disintegration appearing in the secretory cells is due to imperfect fixation and that no necrobiosis of any kind appeared.

The above summary of the evidence for the three theories to account for the introduction of the solids into the milk shows how contradictory is the evidence so far presented. This contradiction, however, is not to be wondered at. The examination of the cells of the actively lactating

mammary gland of a Holstein-Friesian cow showed that they were quite small. Considered in the light of this small size, it is likely that observations on the distal end of a cell might be called by one observer the destruction of this portion and by another the cell in its natural shape. This explanation of the confusion in interpretation of observations in these cells is made further probable by the change of shape which cells undergo at different stages of lactation. Thus, in the mammary gland of a bitch when just emptied, Heidenhain says that the cells were high and columnar and in another bitch where milk had not been drawn for 48 hours the cells were flat. The weakness of the histological evidence is obvious. To the final solution of the problem it appears that other evidence beside the histological observations must be presented.

Some evidence from the physiological side has been presented in the foregoing pages of the interaction of the four variables, age, quantity of milk secreted, diurnal variations, and the effect of the content of one solid on the relation of the other variable, butter fat, or solids-not-fat. These variables give criteria to the efficacy of the three contending hypotheses to explain the release of the milk solids.

Consideration of the manner of metabolism and energy requirement for most bodily functions seems to furnish the explanation of the slight negative correlation of butter-fat concentration with the amount of milk produced, where no such correlation exists with amount of milk production and the other solids. Energy has been shown to be required for most bodily functions. There does not seem to be any reason to suppose that the mammary gland is any exception to this rule when milk is produced. Such energy requirement would of necessity be dependent on the amount of production—that is, the high producer will require more energy and to produce this energy will consequently take a slightly greater amount of fat that might have gone into the milk. The other solids would not be required to furnish any of this energy and consequently would show no effect of the amount of milk produced on their concentration. The correlations obtained show the associations that would be expected with this explanation. The conclusion seems justifiable that the energy required in the production of milk causes a slight reduction in the amount of fat present in the milk of high-producing cows.

The maintenance of the fat concentration of the milk throughout life and the decline of the solids-not-fat apparently represent the normal conditions going on throughout the whole body. It is common knowledge that increase in age generally brings with it a relative decrease in the protein upbuild of the body and an increase in the fat. This increased metabolism of fat appears to extend itself to the mammary gland, as well as to other parts of the body, being just great enough to maintain the butter-fat concentration throughout life, whereas the

relative decreased metabolism of the other solids causes a decrease in the concentration of these solids in the milk.

By far the best evidence yet presented for the secretion theory for the liberation of the milk solids (the third hypothesis) is given by the diurnal variation of the constituents of cow's milk shown above. It is very difficult to see why the cell constitution should change in balance between the solids-not-fat and butter fat between morning and evening milking on any other theory. Why should the cells discharged in the evening contain the ratio a to b of butter fat to solids-not-fat, whereas in the morning the ratio is changed to a markedly lower value of c to b? On the first and second theories the cell must contain a fixed quantity of solids-not-fat, while the butter fat varies in such a way that, in a longer time between the emptying of the gland, the cell accumulates less fat than in the shorter time; or, taken in another way, the cell accumulates relatively more protein as the interval between milkings is lengthened.

Our knowledge of fat formation by other cells of the body makes it probable that either of these two possible alternatives for the formation of this milk fat on the cell-destruction hypothesis are inconsistent with the facts. In the formation of fat, the cell is first composed chiefly of protein material. In this protein material the fat is accumulated in ever-increasing amounts at the expense and crowding out of the protein constituents. This change of the ratio of the fat to solids-not-fat is just opposite to what must take place in the mammary gland on the cell-destruction hypothesis. The ratio of the fat to the solids-not-fat in the fat cells increases as the cells increase in age; the ratio of the fat to the solids-not-fat in milk, derived from the cell breakdown on the two destruction hypothesis, decreases as the age of the cell increases. It is hard to believe that there is such a difference in fat formation going on in the body. It is much more likely that the mechanism of fat formation in the two cases is the same and that the diurnal variation of the ratio of butter fat to solids-not-fat is only another phase of the changes which take place in known secretions. The large variations current in the amount of milk produced, the variations of the constituents with age, the great and characteristic differences in the composition of the milk of two cows of the same breed all add weight to the view that milk is secreted. By analogy, the mammary-gland mechanism for milk secretion would agree with practically all of the glands which secrete. There is no need to assume that rapid cell division is taking place in order to maintain the necessary number of cells for breakdown, as called for on the cell-destruction hypotheses, where no such amount of mitosis as would be necessary is witnessed microscopically. Further, the secretory theory has supporting evidence of whole granules being secreted into the saliva by the salivary glands in much the same way as is done in the secretion of butter fat. The data supporting the secretory hypothesis

are certainly strong, whereas the proof for the cell-destruction hypotheses seems weak when analyzed in the light of the above facts, and, in truth, in some particulars is contrary to known facts. The conclusion that milk is a true secretion seems justified by what we know of the mechanism behind such glands.

The data above presented give us a criterion to judge the value of any hypothesis for the origin of the milk solids from a common mother substance, such as has been suggested by Thierfelder (*31*) and later by Landwehr (*17*), to account for the derivation of casein and lactose from nucleoproteids or glycoproteids of the gland cells by splitting. The correlation of the solids-not-fat and fat might lead one to suppose such a common origin for some component of such solids and the fat. This can not be the case, however, as the correlation of fat and of solids-not-fat with amount of milk and age precludes that possibility, for if such a common origin occurred, the fat and solids-not-fat would necessarily be correlated to these other variables by comparable amounts. The milk components are not correlated equally with either milk quantity or with age; consequently, the hypothesis of a common origin is not tenable.

The correlations furnish the necessary evidence for the determination of an important genetic relation between the hereditary factors for the concentration of the butter fat and the solids-not-fat. Since the data are taken from the presumably homogenous population of the advanced registry Holstein-Friesian cows, linkage of factors for milk solids can not be used to account for this correlation; for, granting the homogeneity of the population, the factors are as likely to be in opposite chromosomes as in the same chromosomes that is, calling one "*a*" and the other "*b*," the parental chromosome content would be:

ab	*a*	*b*	
ab	*ab*	*ab*	*ab*
ab	*a*	*b*	
a	*ab*	*a*	*a*
ab	*a*	*b*	
b	*b*	*b*	*b*
ab	*a*	*b*	

or, reducing down, four chromosomes would be present in equal numbers *ab*, *a*, *b*, . These bred together would give the random distribution, considering that crossing over is as likely to occur, one way as another. This, however, is not what actually happens in our case. The quantity of fat is correlated to the quantity of solids-not-fat per volume of milk. This must mean that some of the factors responsible for fat concentration are responsible for the concentration of sugar, protein, or ash in cow's milk.

From the practical side of increasing the solids content of cow's milk, the importance of the correlation between the butter fat and the solids-not-fat should be pointed out. This high correlation between these variables allows us to use the determination of the percentage content of one as a means of predicting what the content of the other will be. Thus, butter-fat content is easily determined by almost any one familiar with the Babcock test; but solids-not-fat are not so easily determined nor so frequently recorded. We may, in trying to improve the solid content of milk select as breeders those cows which test well with the Babcock apparatus, and at the same time improve the solids-not-fat content of the milk.

SUMMARY

This paper is the fourth of a series of studies on milk now being conducted in the Biological Laboratory of the Maine Agricultural Experiment Station. The data for this study are taken from the semiofficial year record of the pure-bred Holstein-Friesian cows, compiled and supervised by the Holstein-Friesian Association.

(1) The means, standard deviations, and coefficients of variation are given for these year records. The mean annual production of these animals was 15,417 pounds of milk, 528 pounds of butter fat, 1,303 pounds of solids-not-fat at a mean age of four years. The standard deviations are 3,742 pounds of milk, 134 pounds of butter fat, 260 pounds of solids-not-fat, and two years. The coefficients of variations are, respectively, 24, 25, 20, and 50 per cent.

(2) Comparison is made of Holstein-Freisian milk with the milk of other breeds and other species.

(3) Correlations are presented between the variables butter-fat percentage and amount of milk produced, butter-fat percentage and age at test, solids-not-fat percentage and amount of milk, and solids-not-fat and age at test. These correlations lead to the following conclusions: (a) As the amount of milk given by the cows in this test increases, the percentage composition of butter fat decreases. The amount of this decrease is highly significant, measured statistically. Considered practically, this fall in butter-fat content would not be easily detected in small samples. (b) The correlation between the age at test and butter fat is not significant. (c) The correlation between the amount of milk produced and the percentage of solids-not-fat is not significant; or, put in another way, the quantity of milk produced for one year is independent of the concentration of the solids-not-fat. This, from a genetic viewpoint, means that the hereditary factors for high or low milk production are separate and distinct from those causing a high percentage of solids-not-fat. (d) The correlation of age at test and solids-not-fat is -0.2191 ± 0.0351—that is, as the age of a cow increases, the solids-not-fat percentage of the milk decreases. (e) The constants for the linearity of regression are given. They show the regressions to all be linear. (f)

These conclusions give us two variables which influence the concentration of butter fat and solids-not-fat differently. This difference in action of these variates proves that the butter fat and the solids-not-fat can not have a common mother chemical from which they are derived from splitting.

(4) Correlations are presented between the variables, pounds of milk, butter fat, and solids-not-fat. Each variable is highly correlated, the correlation ranging from $r=0.8644 \pm 0.0093$ to $r=0.9497 \pm 0.0036$. In each case the regressions are linear. The partial correlation between butter fat and solids-not-fat for a constant value of the milk is found to be 0.5635 ± 0.0252. This correlation, together with those above, furnishes the data necessary to establish the conclusion that some of the factors responsible for high concentration of butter fat are also responsible for high concentration of some of the solids-not-fat in cow's milk. Another important practical conclusion may be drawn from this correlation; that if it is desired to improve either the butter fat or solids-not-fat concentration in a given herd the determination of the concentration of either solid will also result in an increased concentration of the other solid.

(5) Data on the diurnal variation of cow's milk are presented. These data show that the morning milk is between 0.678 and 0.723 per cent lower in butter fat than in the evening milk throughout the whole lactation. No appreciable difference occurs in the solids-not-fat. These data offer criteria between the theories to account for the secretion of the milk solids. In the cell-disintegration theories the cell must contain a fixed quantity of solids-not-fat, while the butter fat varies so that in the longer interval between milkings the cell accumulates less fat than in the short time; or, taken the other way, the cell contains relatively more protein and sugars than fat as the interval between milkings lengthens. This is contrary to our knowledge of fat formation, for it is commonly accepted that first comes the cells composed largely of protoplasm and that as time goes on this cell is more and more loaded with fat at the expense of the protoplasm. Unless these mammary cells behave very differently in the formation of this fat than other body cells, this variation is enough to discredit seriously the hypothesis of cell disintegration to account for these milk solids; and in fact, to make it an absurdity. Furthermore, so far as our knowledge of the variations of secretory glands goes, the variations of the milk fall in well with the secretory hypothesis to account for these solids.

LITERATURE CITED

(1) Ashcroft, William.
1905. THE MILKING TRIALS OF 1904. *In* Jour. Brit. Dairy Farmers' Assoc. v. 19, p. 88–117.

(2) BERTKAU, F.

1907. EIN BEITRAG ZUR ANATOMIE UND PHYSIOLOGIE DER MILCHDRÜSE. *In* Anat. Anz., Bd. 30, No. 7/8, p. 161–180, 7 fig. Literatur, p. 179–180.

(3) BLAKEMAN, John.

1905. ON TESTS FOR LINEARITY OF REGRESSION IN FREQUENCY DISTRIBUTIONS. *In* Biometrika, v. 4, pt. 3, p. 332–350.

(4) BROUHA.

1905. RECHERCHES SUR LES DIVERSES PHASES DU DÉVELOPPEMENT ET DE L'ACTIVITÉ DE LA MAMELLE. *In* Arch. Biol., t. 21, p. 459–603, pl. 18–20. Travaux renseignes, p. 591–596.

(5) BUTTENBERG, P., and TETZNER, F.

1904. EIN BEITRAG ZUR KENNTNIS DER ZIEGENMILCH. *In* Ztschr. Untersuch. Nahr. u. Genussmtl., Bd. 7, p. 270–272.

(6) ELSDON, G. D.

1916. NOTE ON HUMAN MILK. *In* Analyst, v. 41, no. 480, p. 74.

(7) FARRINGTON, E. H.

1905. DAIRY COW DEMONSTRATION OF THE LOUISIANA PURCHASE EXPOSITION. 64 p. [Fort Atkinson, Wis.]

(8) FISCHER, K.

1908. ÜBER ZIEGENMILCH UND ZIEGENBUTTER. *In* Ztschr. Untersuch. Nahr. u. Genussmtl., Bd. 15, Heft 1, p. 1–13.

(9) FULLER, J. G., and KLEINHEINZ, Frank.

1904. ON THE DAILY YIELD AND COMPOSITION OF MILK FROM EWES OF VARIOUS BREEDS. *In* Wis. Agr. Exp. Sta. 21st Ann. Rpt. [1903]/04, p. 48–50.

(10) FULLER, Valancey E.

1901. PAN-AMERICAN MODEL DAIRY. FINAL REPORT. Jersey Advocate and Dairyman, v. 1, no. 37, Sup. 2 p.

(11) GAVIN, William.

1913. STUDIES IN MILK RECORDS: ON THE ACCURACY OF ESTIMATING A COW'S MILKING CAPABILITY BY HER FIRST LACTATION YIELD. *In* Jour. Agr. Sci., v. 5, pt. 4, p. 377–390.

(12) HAECKER, A. L.

1907. DAIRY HERD RECORD FOR TEN YEARS. *In* Nebr. Agr. Exp. Sta. Bul. 101, p. 1–27, fig. 1–5.

(13) HEIDENHAIN, R.

1883. DIE MILCHABSONDERUNG. *In* Hermann, Handbuch der Physiologie, Bd. 5, F. 1, p. 380.

(14) HOFMANN, K., and HANSEN, J.

1911. LEISTUNGSPRÜFUNGEN MIT VERSCHIEDENEN RINDERSCHLÄGEN. *In* Landw. Jahrb., Bd. 40, Ergänzungsbd. 1, p. 210–305, 345–430, 14 pl. Abstract in Exp. Sta. Rec., v. 26, no. 9, p. 879–880. 1912.

(15) HOLSTEIN-FRIESIAN ASSOCIATION OF AMERICA.

1907–17. Advanced Registry Year Book. v. 18–28.

(16) INGLE, Herbert.

1903. VARIATIONS IN THE COMPOSITION OF COW'S MILK. *In* Trans. Highland and Agr. Soc. Scotland, s. 5, v. 15, p. 135–182.

(17) LANDWEHR, Herm. Ad.

1887. UEBER DIE BEDEUTUNG DES THIERISCHEN GUMMIS. *In* Arch. Physiol. [Pflüger], Bd. 40, p. 21–37.

(18) LEATHER, J. Walter

1901. THE COMPOSITION OF INDIAN COWS AND BUFFALOES MILK. *In* Analyst, v. 26, no. 299, p. 40–42.

(19) LLOYD, Fred J.

1904. THE MILKING TRIALS OF 1903. *In* Jour. Brit. Dairy Farmers' Assoc., v. 18, p. 95–121.

(20) PEARL, Raymond.
1914. ON THE LAW RELATING MILK FLOW TO AGE IN DAIRY CATTLE. *In* Proc. Soc. Exp. Biol. and Med., v. 12, no. 1, p. 18-19.

(21) PEARSON, Karl.
1896. MATHEMATICAL CONTRIBUTIONS TO THE THEORY OF EVOLUTION. III. REGRESSION, HEREDITY, AND PANMIXIA. *In* Phil. Trans. Roy. Soc. London, s. A, v. 187, p. 253-318.

(22) ———
1905. MATHEMATICAL CONTRIBUTIONS TO THE THEORY OF EVOLUTION. XIV. ON THE GENERAL THEORY OF SKEW CORRELATION AND NONLINEAR REGRESSION. Drapers' Co. Research Mem. Biom. Ser. 2, 54 p., 3 pl.

(23) ———
1910. NOTE ON THE SEPARATE INHERITANCE OF QUANTITY AND QUALITY IN COW'S MILK. *In* Biometrika, v. 7, pt. 4, p. 548-550.

(24) RICHMOND, H. Droop.
1910. THE COMPOSITION OF MILK. *In* Analyst, v. 35, no. 411, p. 231-237.

(25) SANNA, Andrea.
1905. COMPOZIONE QUANTITATIVA MEDIA DEL LATTE PECORINO DELLE CAMPAGNE MERIDIONALI DELLA SARDEGNA. *In* Staz. Sper. Agr. Ital. v. 38, fasc. 4, p. 289-292.

(26) SCHLOSSMANN, ARTHUR.
1897. UEBER ESELSMILCH. *In* Ztschr. Physiol. Chem., Bd. 23, Heft 3, p. 258-264.

(27) SHEPPARD, W. F.
1907. THE CALCULATION OF MOMENTS OF A FREQUENCY DISTRIBUTION. *In* Biometrika, v. 5, pt. 4, p. 450-459.

(28) STEINHAUS, Julius.
1892. DIE MORPHOLOGIE DER MILCHABSONDERUNG. *In* Arch. Anat. u. Physiol., 1892, Sup. Bd. Physiol. Abt., p. 54-68, pl. 5-7.

(29) SUTHERST, Walter F.
1902. THE COMPOSITION OF COLOSTRUM. *In* Chem. News, v. 86, no. 2223, p. 1-2.

(30) SVOBODA, H.
1904. VERGLEICHENDE UNTERSUCHUNGEN ÜBER DIE BESCHAFFENHEIT UND MENGE DER MILCH DER BEIDEN KÄRNTNER HAUPTLANDESRASSEN. *In* Oesterr. Molk. Ztg., Jahrg. 11, No. 14, p. 191-193; No. 15, p. 205-207.

(31) THIERFELDER, Hans.
1883. ZUR PHYSIOLOGIE DER MILCHBILDUNG. *In* Arch. Physiol. [Pflüger], Bd. 32, p. 619-625.

(32) TRILLAT and FORESTIER.
1902. SUR LA COMPOSITION DU LAIT DE BREBIS. *In* Jour. Agr. Prat., ann. 66, sem. 2 (n. s. t. 4), No. 28, p. 38-39.

(33) VIETH, P.
1885. ON THE COMPOSITION OF MARES' MILK AND KOUMISS. *In* Analyst, v. 10, Dec., p. 218-221.

(34) VIGOR, H. D.
1913. THE CORRELATION BETWEEN THE PERCENTAGE OF MILK FAT AND THE QUANTITY OF MILK PRODUCED BY AYRSHIRE COWS. *In* Sup. Jour. Bd. Agr. [London], 11, 28 p.

(35) WHITLEY, S. R.
1906. THE MILKING TRIALS. *In* Jour. Brit. Dairy Farmers' Assoc., v. 20, p. 135-169.

(36) ———
1909. THE MILKING TRIALS OF 1908. *In* Jour. Brit. Dairy Farmers' Assoc., v. 23, p. 101-144.

(37) WILSON, James.
1910. THE SEPARATE INHERITANCE OF QUALITY AND QUANTITY IN COWS' MILK. *In* Sci. Proc. Roy. Dublin Soc., n. s. v. 12, no. 35, p. 470-479.

(38) WINDISCH, Richard.
1904. BEITRÄGE ZUR KENNTNIS DER BÜFFELMILCH. *In* Ztschr. Untersuch. Nahr. u. Genussmtl., Bd. 8, Heft 5, p. 273-278.

(39) WOLL, F. W.
1899. THE COMPOSITION OF SOW'S MILK. *In* Wis. Agr. Exp. Sta. 16th Ann. Rpt. [1898]/99, p. 267-270.

(40) ———
1901. ON THE AVERAGE COMPOSITION OF MILK OF PURE-BRED COWS OF DIFFERENT BREEDS. *In* Wis. Agr. Exp. Sta. 18th Ann. Rpt. [1900]/01, p. 85-97.

NEW BIOLOGIC FORMS OF PUCCINIA GRAMINIS[1]

[PRELIMINARY PAPER]

By E. C. STAKMAN, *Head of Section of Plant Pathology, Department of Plant Pathology, Botany, and Department of Agriculture, University of Minnesota;* M. N. LEVINE, *Field Assistant, Bureau of Plant Industry, United States Department of Agriculture;* and J. G. LEACH, *Shevlin Fellow, University of Minnesota*

COOPERATIVE INVESTIGATIONS BETWEEN THE AGRICULTURAL EXPERIMENT STATION OF THE UNIVERSITY OF MINNESOTA AND THE BUREAU OF PLANT INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE

Several biologic forms of *Puccinia graminis* on wheat (*Triticum* spp.) have been described. Originally *P. graminis tritici* was supposed to be the only form capable of attacking wheat varieties. None of the common wheats (*T. aestivum*) was resistant to this form, although several varieties of durum (*T. durum*), emmer (*T. dicoccum*), and einkorn (*T. monococcum*) were either resistant or almost immune.

The first demonstration that there was more than one form of stemrust on wheat was made in 1916 when *P. graminis tritici-compacti* was described.[2] This form proved to be especially interesting and significant because it could not infect the hard spring wheats normally, but developed well on soft wheats. It was also found that many of the hard winter wheats were resistant to the new form. The range of parasitism of the second form was therefore narrower than that of the ordinary *P. graminis tritici*. But in 1918 Melchers and Parker[3] found that Kanred, Kansas P. 762 (CI 5146),[4] Kansas P. 1066 (CI 2879), and Kansas P. 1068 (CI 5880), three selections from the Crimean group made at the Kansas Experiment Station, were almost immune to *P. graminis tritici*. These selections were also found to be moderately resistant to *P. graminis tritici-compacti*, and they therefore seemed to be resistant to the stemrust of wheat. Later Melchers and Parker[5] found a form which infected Kanred and the two other selections normally. Levine and Stakman[6] and Leach[7] had begun an intensive study of the parasitic capabilities of forms of *P. graminis* on varieties

---

[1] Published, with the approval of the Director, as Paper 144 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] STAKMAN, E. C., and PIEMEISEL, F. J. A NEW STRAIN OF PUCCINIA GRAMINIS. (Abstract.) *In* Phytopathology, v. 7, no. 1, p. 73. 1917.

[3] MELCHERS, Leo E., and PARKER, John H. THREE VARIETIES OF HARD RED WINTER WHEAT RESISTANT TO STEM RUST. *In* Phytopathology, v. 8, no. 2, p. 79. 1918.

[4] CI = Cereal Investigations.

[5] MELCHERS, Leo E., and PARKER, John H. ANOTHER STRAIN OF PUCCINIA GRAMINIS. Kans. Agr. Exp. Sta. Circ. 68, 4 p. 1918.

[6] LEVINE, M. N., and STAKMAN, E. C. A THIRD BIOLOGIC FORM OF PUCCINIA GRAMINIS ON WHEAT. *In* Jour. Agr. Research, v. 13, no. 12, p. 651–654. 1918.

[7] LEACH, J. G. A COMPARATIVE STUDY OF THE PARASITISM OF PUCCINIA GRAMINIS TRITICI AND PUCCINIA GRAMINIS TRITICI-COMPACTI. To be published as master's thesis, University of Minnesota.

of wheat and other species of Triticum, and also found a form which developed normally on Kanred, P. 1066, and P. 1068. It was clear from these results that the new forms could therefore do something which neither of the two other forms could do.

But none of the known forms could infect White Spring emmer (Minnesota 1165), durum (Mindum, CI 5296), and several other varieties, mostly durums. It was perfectly evident from the work with *P. graminis tritici-compacti* [1] and the two other new forms that the biologic specialization within the genus Triticum could only be determined by testing many species and varieties and that there was a strong probability that forms of rust would be found which were capable of attacking varieties resistant to all known forms of stemrust. This is exactly what has been found. A form was found which infected White Spring emmer and Mindum normally. The work has continued until about a dozen forms have been found up to the present time (Oct. 1, 1918).

About 25 varieties and strains of *Triticum aestivum, T. durum, T. compactum, T. dicoccum,* and *T. monococcum* are being used as differential hosts, and no variety so far tried is resistant to all of the rust forms except Khapli (CI 4103), an emmer originally imported from India. Some of the forms are very virulent on many varieties, while others are weak and can attack only a few varieties successfully. Some forms differ from each other only in their action on one or two varieties; but these differences are definite and consistent. No attempt has yet been made to name the recently discovered forms.

The factors governing the distribution of the forms are not at all clear. Material has been collected from 27 States and most sections of the country are represented. Two distinct forms have often been isolated from the same lot of material, and at least four have been found in Minnesota.

The fact that there are so many biologic forms of stemrust on wheat seems to be of profound significance in at least two ways. It is an additional reason for eradicating the rust-susceptible varieties of barberry (*Berberis* spp.), and it is of the greatest importance in the work of breeding wheats for rust resistance.

Many of the virulent forms seem to occur in the Northern States, where everyone will now concede that the barberry is of tremendous importance in the persistence of stemrust from year to year. Eradicate the common barberry and there is reason to believe that these forms may gradually die out entirely, or at least be reduced to a condition bordering on impotence. The fact also that in the South and on the Pacific coast, where barberry does not rust commonly, the forms of rust seem to be more uniform than in other regions certainly lends some color to the view that the barberry may have some effect on their development. A hypothesis that forms may have originated by hybridiza-

[1] LEACH, J. B. OP. CIT.

tion on the barberry may be worth investigating. Of course mutation and adaptation must be considered also in any attempt to explain how so many forms originated.

The fact that the same variety of wheat may be immune in one locality and susceptible in another is clearly explained. Formerly recourse was taken to the theory that the environmental conditions changed the physiologic processes and materials of wheat varieties so fundamentally that the resistance of the plants broke down. The real explanation of this phenomenon however is the fact that there are many biologic forms of the rust fungus. This has actually been demonstrated in field experiments. There is also preliminary evidence to show that the same thing may be true of *Puccinia triticina* on wheat.

Methods for breeding for rust resistance must be changed fundamentally—if indeed it is worth while to do such work at all until more is known about the specialization of the rust fungus. The breeder must know and work with those forms of rust which occur in the region for which his new variety is intended; and even then breeding must be very largely a regional or even a local problem. For instance, in the breeding plots at the Minnesota Agricultural Experiment Station certain varieties were practically immune to stemrust, but rust forms have been found within 50 miles of the plots which can attack these varieties so heavily as to make them worthless for rust resistance.

The discovery of so many forms of stemrust on wheat complicates the rust problem seriously. Extensive experiments are under way to determine the number, characteristics, and distribution of biologic forms as well as their constancy and probable origin.

ADDITIONAL COPIES
OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
5 CENTS PER COPY
SUBSCRIPTION PRICE, PER YEAR, $3.00